

Plant Gene Register

The Two *psbA* Genes from the Thermophilic Cyanobacterium *Synechococcus elongatus*¹

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The D1 protein, one of the constituent proteins of the PSII reaction center complex, forms a heterodimer with D2 protein and carries the prosthetic components functioning in the primary charge separation (Nanba and Satoh, 1987). It is also known to be the protein most rapidly degraded in the thylakoid membranes (Ellis, 1981). Here we report the nucleotide and deduced amino acid sequences of the two continuous *psbA* genes encoding D1 proteins from the thermophilic cyanobacterium *Synechococcus elongatus* (Hirano et al., 1980) (Table I).

Analysis of a sequence of 3183 bp showed two open reading frames. Each of the two *psbA* genes, *psbA1* and *psbA2*, has a “–10” and a “–35” prokaryotic promoter-like sequence and a putative terminator region, suggesting independent transcription of the genes. The nucleotide sequence of the *psbA1* gene has been reported (Kloos et al., 1993), but our sequence differs in five nucleotides or in one amino acid residue (Thr¹³⁴ replaced by Ser¹³⁴) from that reported previously. Each gene product is composed of 360 amino acid residues and the distance between the termination codon TAA of *psbA1* gene and the initiation codon ATG of *psbA2* gene is only 316 bp. Such close association of *psbA* genes has not been found in other organisms. The deduced amino acid sequences of the two gene products are different in 34 residues.

We have isolated the D1 protein and analyzed the interior amino acid sequence of the protein, because the N-terminal amino acid was blocked. The protein was digested with lysylendopeptidase and the N-terminal sequence of one peptide was analyzed. The sequence of 16 residues perfectly coincides with region of the deduced amino acid sequence that starts from a residue that is unique to the product of the *psbA1* gene (Lys³¹⁰). This suggests that the *psbA1* gene is expressed mainly in the cyanobacterial cells.

The amino acid sequences of the *Synechococcus* genes are 79 to 89% homologous to the *psbA* genes of other cyanobacteria (Osiewacz and McIntosh, 1987; Ravnikar et al., 1989; Vrba and Curtis, 1989; Mets et al., 1990) and higher plants (Zurawski et al., 1982; Sugita and Sugiura, 1984). There are,

Table I. Characteristics of two *psbA* genes from *S. elongatus*

Organisms:
<i>Synechococcus elongatus</i> (Thermophilic cyanobacterium).
Cellular Localization:
Thylakoid membrane.
Function of Gene:
<i>psbA</i> encodes a protein that binds prosthetic components functioning in the primary charge separation in PSII.
Techniques:
A 4.0-kb <i>EcoRI</i> / <i>HindIII</i> fragment containing two <i>psbA</i> genes was isolated from the λ ZAPII size fraction genomic library with an amplified 0.9-kb DNA fragment as a probe. The nucleotide sequences of both strands were determined by a modified dideoxy chain termination method with an Applied Biosystems 373 DNA sequencer.
Sequence Identification:
Comparison of the deduced amino acid sequences with sequences from other D1 proteins.
Feature of the Gene Structure:
Two <i>psbA</i> genes have open reading frames of 1083 bp. The distance between the <i>psbA</i> genes is 316 bp. Each of the genes has a “–10” and a “–35” prokaryotic promoter-like sequence and a putative termination region.
Features of the Deduced Amino Acid Sequences:
The deduced amino acid sequences of two <i>psbA</i> genes contain 360 amino acids and are 79 to 89% identical with D1 proteins from other organisms. The two genes products are different in 34 residues.
Expression of the Genes:
A lysylendopeptidase-digested fragment was completely identical with the sequence of the <i>psbA1</i> gene product, which suggests that the <i>psbA1</i> gene is expressed mainly in the cyanobacterial cells.

however, notable amino acid replacements (Cys²¹² and Lys³¹⁰ in the *psbA1* gene; His³⁰⁴ and Asn³¹² in the product of the two genes), which might be important for the thermostability of the *Synechococcus* protein. A replacement of Ser or Ala²¹² by Cys in the *psbA1* gene product has been suggested to contribute to the stability of the PSII reaction center (Kloos et al., 1993). No corresponding replacement was found in the *psbA2* gene product. Another notable feature of the deduced sequence of the thermostable protein is a cluster of variations in the C-terminal sequence: His³⁰⁴ and Asn³¹² are common to the products of the two genes, and Lys³¹⁰ is unique to the *psbA1* gene product.

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